Some Notes on the Gametogenesis of Ornithorhynchus Paradoxus.

By

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With Plates 12, 13, 14. and 1 Text-figure.

Contents.

1. Introduction.

In this paper I have described in as much detail as was possible the oogenesis of the duck-billed platypus of Australia. Owing to the unique position of the Prototheria, any new facts with regard to their germ-cells is sure to be of value. I must take this opportunity of thanking Professor J. P. Hill, F.R.S., for allowing me to study his material of Ornithorhynchus, without which I could not have published these notes.

There is no account extant of the detailed structure of the ovarian egg of Ornithorhynchus, of the yolk formation, of the maturation stages, or of the corpus luteum. Such accounts of the ovary as are published are scrappy and full of errors, this, however, being chiefly due to the scanty and poor material at the disposal of the various observers who have attacked these problems. The material at my disposal, while not having been prepared by the most modern technique, is well preserved by routine methods and allows of a fuller description of various problems than hitherto given. The material consisted of one ovary preserved in Flemming's strong fluid, and of several ovaries preserved by a variety of picric and bichromate fixatives. Most of the new results were procured by examination of the Flemming-fixed ovary.

This work was partly carried out in the Embryological Laboratory, University College, London, and was finished in the Zoological Laboratory, Dublin University. Apart from his kindness in lending me the material, I have to thank Professor J. P. Hill for assisting me by lending some of the literature on Ornithorhynchus and Echidna.

2. Previous Work on Gametogenesis of Ornithorhynchus and of Echidna.

Thirty-seven years ago E. B. Poulton, in his paper on 'The Structures connected with the Ovarian Ovum of Marsupialia and Monotremata', gave some account of the general appearance of the ovary and follicle of Ornithorhynchus and Echidna. Poulton's material consisted only of ovaries removed from spirit specimens, and he was consequently much handicapped. Nevertheless, he succeeded in establishing several facts of great importance. The ovary of Ornithorhynchus, according to Poulton, is flat or compressed, oval, and about 13 mm. long, 7 mm. wide, and 2 mm. thick. The follicles are confined to the edge of the transverse section of the ovary, i.e. on the surface of the ovary; there does not seem to be any distinct arrangement of follicles, according to size, but the small ones always seem to be near the surface. Poulton noticed that there was evidence that the large follicles were constricted off, in the presence of a deep furrow encircling some of them. By this I believe he means that the egg (and follicle) is constricted from outside, and tends to hang somewhat freely on the surface of the ovary.

Poulton identified a follicular epithelium, which he considered to be of one layer, 'the whole of the time the ovum remains in the follicle'.

This author also describes faithfully the zona pellucida, follicle, basement membrane, and tunica fibrosa, and establishes the fact that the 'ova of Monotremes practically fill their follicles, and are of considerable size'. The nucleus Poulton considered to be central in the small ova. He recognizes in the older egg a peripheral stainable granular area, and, deeper down, a lighter granular area, beneath which lies the yolk.

It is remarkable that Poulton should have been able to describe so many interesting facts from such poor material.

Three years later, in 1887, Caldwell published a paper on 'The Embryology of the Monotremata and Marsupialia', in which he pointed out that Poulton and Guldberg had wrongly stated that the follicular epithelium remains always a single layer of cells.

Guldberg and Beddard both described the ovary of Echidna. They showed that it resembled in its oogenesis the condition already described by Poulton for Ornithorhynchus.

Probably the finest collection of Monotreme material is that procured by Semon about 1893; this observer had at his disposal a large number of eggs in all stages. He gives no account of the oogenesis, and his description of the structure of the egg consists of thirty-five lines of general comment, without any detailed account of his material. It is therefore difficult to know how much Semon understood of the structure of the egg. Certain appearances drawn in his figures of the egg are undescribed in the text. In some cases it is impossible to know whether Semon's figures of supposed nuclei are cells or nucleoli; this applies especially to his Tafel IX, figuring early stages of development.

Writing of the full-grown egg, Semon says: 'Die Keimscheibe ruht auf einem Lager von feinkörnigem, weissem Dotter, und dieser entsendet nach innen eine strangförmige Fortsetzung, einen "Dotterstiel", der im Centrum sich flaschenförmig zu einer Latebra aufbläht. Die Elemente des gelben Dotters sind kugelrund; gegen den weissen Dotter zu, besonders in der Gegend der Keimscheibe, nimmt der Durchmesser der Kugeln des gelben Dotters continuirlich ab. An der Grenze erblickt man häufig die Kugeln des gelben Dotters in allen Stadien des Zerfalls zu kleineren und kleinsten Elementen. In gleichem Maasse wie das Blastoderm den Dotter umwächst, breitet sich an der Oberfläche des letzteren und ersteren eine Schicht von weissem Dotter aus.'

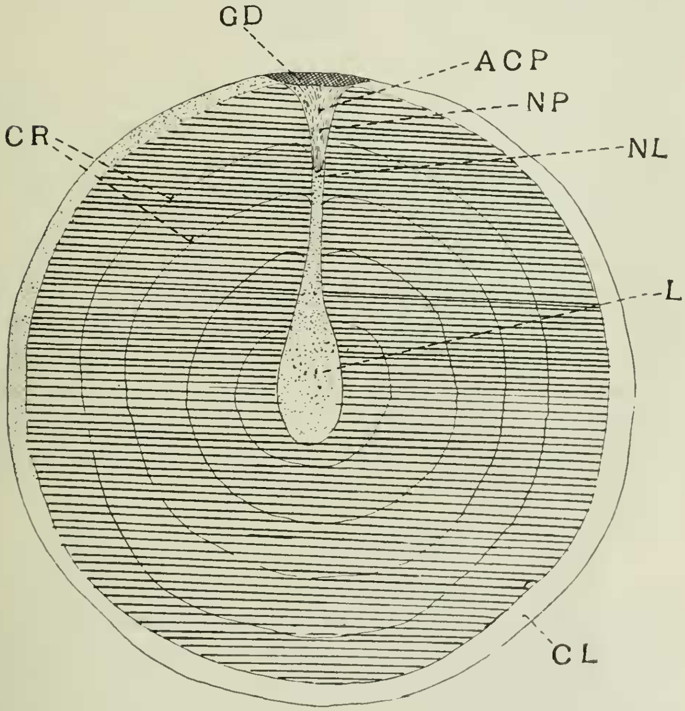
In his figures of sections of the eggs of both Echidna and Ornithorhynchus, Semon draws, within the more central part of the cross-section of the egg, from one to as many as three concentric rings lying in the yellow yolk, as is well known to occur in the hen's egg, but he does not describe these rings in his text. His description of the structure of the egg is very poor. It would probably be worth while for a capable cytologist to re-describe the sections of eggs of both Echidna and Ornithorhynchus procured by Semon's party in Australia.

3. General Note on the Structure of the Egg of the Sauropsida.

In both the Aves and the Reptilia, the egg, as is well known, has a very complicated structure, and for the purpose of comparison with that of Ornithorhynchus I have given a diagram in Text-fig. 1.

The germinal disk (GD) is formed of pure protoplasm free of any but the smallest yolk-spheres; this protoplasmic disk contains a very granular, generally somewhat basophil, type of protoplasm, which can readily be distinguished from the clear cone of protoplasm (ACP) which lies below. This clear cone of protoplasm is not granular, and passes insensibly into the disk above, on the one hand, and into the neck of the latebra (NL) below, on the other hand (Nucleus of Pander). The latebra (L) is formed of a clear substrate containing numbers

TEXT-FIG. 1.



of fine yolk-granules. Completely surrounding the egg, and forming a peripheral area, is a thin layer of clear protoplasm containing very fine yolk-spheres (CL). All the internal substance of the egg, excepting that part occupied by the latebra (L), is filled with enormous numbers of large coarse yolk-spheres; and within this substance can be found concentric rings of clear material (CR) which are said to mark areas of growth of the yolk (see Riddle, **5**).

The peripheral clear area (CL), the cone of protoplasm (ACP), and the latebra are generally described as containing white yolk-spheres, the rest of the egg mainly yellow yolk-spheres.

The clear thin layers of concentric stratification (CR) have been said to contain white yolk-spheres, though this has not been settled satisfactorily. Riddle (**5**), however, believes that the concentric layer does contain white yolk, and is a growth-mark.

Semon's description of the yolk of the egg of Ornithorhynchus does not include any mention of these concentric layers of stratification within the egg, but in his figures he shows eggs of Echidna and Ornithorhynchus which contain one (Tafel VIII, fig. 23), two (fig. 25 and fig. 19), and three (fig. 20) layers, as depicted in Text-fig. 1 (CR) of this paper.

It is possible that the egg of Ornithorhynchus might contain these concentric lines of growth, if such they be. The varying number of lines are probably significant of the different periods or epochs of the year during which the eggs grew most; that with two rings possibly grew in two sudden well-marked periods, and so on. This opinion is supported by Riddle's work on feeding Sudan III to laying fowls (**5**).

4. General Account of the Formation of Egg-membranes in Sauropsida and Mammalia.

In a recent paper (**8**) Miss Alice Thing has studied the formation of the zona pellucida in various turtle eggs. When the young oocyte of the turtle has reached a size two or three times that of the oogonium, it becomes surrounded by a flattened epithelium which persists as one layer throughout the course of development of the egg. With the gradual growth of the oocyte, the epithelial cells take on a definite prismatic shape and increase in height in the axis perpendicular to the surface of the egg. Occasional mitoses prove, that to accommodate the increasing volume of the egg, the epithelium extends itself by division of its constituent cells; in very large eggs numerous mitoses occur. The epithelial cells forming the follicle are sharply marked off from one another by intercellular channels filled with intercellular substance. The latter undergoes early a change of constitution and becomes transformed at the level of the surface of the cells into the special cement known as the terminal bars.

The zona pellucida is formed by two or three different elements. It takes its origin as a veil-like formation consisting of a mosaic of terminal bars and polygonal fields within which may be recognized the small, pale areas, future canals of the adult membrane separated by pale and dark filaments giving origin to the future fundamental substance of the adult membrane. The fundamental substance of the zona pellucida is developed as a cuticular element, by the terminal bars or primary network, that is by a definite special intercellular cement possessing the property of extension over the free surface of the epithelial cells and forming connexions there with the delicate secondary network apparently produced directly by the superficial cytoplasm of the epithelial cells.

With regard to the origin of the zona pellucida in mammals, I believe that there are three possible methods of development: the zona might develop from the follicular epithelium, it might develop from the egg-cytoplasm, or it might be developed under the influence of, and from, both egg-cytoplasm and follicular epithelium.

The majority of present-day workers appear to believe that the zona of Mammalia develops from the follicular epithelium alone. This is the view of such well-known older observers as Flemming, Retzius, Fischer, Von Ebner, Bonnet, and Rubaschkin. But Van Beneden, Sobotta, Waldeyer, and Kolliker all believe that the zona pellucida is secreted by the egg-cytoplasm. In support of this view are such observations as that of Van Beneden, who described in a bat the fact that while there may be two eggs in such close contact that at one place the follicle is interrupted, yet at this region the zona is properly developed. This is not of very rare occurrence in ovaries of placentals, and is certainly difficult to explain if one believes that the zona is of purely follicular origin.

5. General Account of the Yolk Formation in Birds and Amphibia.

In both birds and amphibians the egg is richly provided with yolk, i. e. macrolecithal. The formation of yolk in the egg of amphibians does not seem to have been followed out with any detail or pains by a modern worker, and though I have made numerous preparations by the best methods, it has been difficult to determine the exact source of origin of the yolk-spheres (see Gatenby, **15**, p. 139).

In the amphibian oogenesis the mitochondria spread out mainly to form a deep cortical zone on the periphery of the egg. It is in this zone that the first sign of yolk-granules appears, but it is wellnigh impossible to give an opinion as to whether the yolk originates directly from the mitochondria, or whether the latter only elaborate materials which, precipitating in the ground cytoplasm, come to form the separate spheres of substance we recognize as yolk.

Van Durme, in his monumental work on the oögenesis of birds (**10**), has entered into the subject with care, and has produced a paper which may be accepted as an authentic account of the steps in the formation of yolk in birds. He recognizes in the oocyte just before the beginning of the extensive yolk formation: (1) an attraction sphere containing a centrosome, (2) a yolk-forming region or vitellogenous cloud, (3) a quantity of fatty yolk. The vitellogenous cloud is formed of mitochondria of various types, e. g. chondriomites, chondriosomes, and it soon undergoes a process of dissociation. This dissociation of the ‘ couche vitellogène ’ invokes the appearance throughout the egg-cytoplasm of a uniform layer of mitochondria. This uniformity does not last long, for soon afterwards three distinct mitochondrial zones appear; a cortical dense zone, an inner deeper, and an internal still deeper zone.

The first vestiges of yolk formation are the appearance of clear yolk-vesicles (vacuoles) in the neighbourhood of the cortical fatty layer, thus constituting a peripheral vacuolated area, which spreads gradually towards the centre of the yolk;

a second vacuolated area appears around the nucleus, known as the perinuclear vacuolated zone. These two zones meet at the animal pole of the egg, above the nucleus, forming the vacuolated nuclear cap.

Subsequently in this second phase of vitellogenesis the first true yolk-spheres put in an appearance firstly in the region of the exoplasm, then more deeply in the endoplasmic region. Van Durme unhesitatingly states that these yolk-spheres partly arise from the larger mitochondria, and partly from the contents of the clear yolk-vesicles (vacuoles).

From this stage onwards the more deeply-lying mitochondria become fewer, the yolk-elements more numerous, but the cortical mitochondrial zone persists throughout all stages.

6. The Structure of the Ovary of Ornithorhynchus.

On taking up a slide of sections of the ovary of Ornithorhynchus and examining it with the naked eye, one is first of all struck by the enormous size of the riper eggs. These are much larger than the full-grown ovarian oocytes of the frog, and of course infinitely larger than those of a rabbit or dog. As in the ovary of a Sauropsidan, the eggs project out around the surface of the organ in a way familiar to any one who has examined the ovary of a fowl or turtle. Thus, while the eggs may be very large, the stroma and general extent of the whole ovary is relatively small. This will be best understood by reference to Pl. 12, fig. 3: in this ovary there was at least one egg nearly if not quite ripe (o), which measured 4·36 mm. in diameter in its shortest way, by 4·52 mm. in its longest way.

In the ovary drawn in Pl. 12, fig. 3, no corpora lutea were to be found, and when these occur they protrude from the surface of the ovary almost as much as the full-grown egg. In several of the ovaries I have examined there are two corpora lutea close together, and these form by far the most prominent structures in the ovaries in question.

Examined under the low power of a microscope the most striking features of the Ornithorhynchus ovary are the innumerable lacunae or spaces in the ground-work or stroma. The biggest of these spaces are drawn in Pl. 12, fig. 3, being cross-hatched (CA), but to gain a better understanding of this peculiarity one must examine fig. 4 of Pl. 13. Here the extraordinary structure of the ovary is demonstrated, a well-marked germinal epithelium is recognizable (GE), and beneath it are a row of oocytes in various stages; on the right of Pl. 13, fig. 4, the oocytes are found to lie in a more solid cortical area of the ovary, which is marked off at this region quite sharply by the wide and numerous lacunae, with their trabeculae in between (TR). These cavities do not contain blood, or lymph corpuscles, but seem to have been occupied by a non-corpuscular fluid, which leaves no trace of coagulum in the finished sections.

As the young oocytes grow older they tend to become completely surrounded by strands of much vacuolated tissue, as is indicated in the largest oocyte drawn in Pl. 13, fig. 4. This feature is certainly one of the most remarkable in the ovary of Ornithorhynchus. It will therefore be clear that by the time an egg has reached the stage drawn in Pl. 13, fig. 4 (roughly one-eighth of its full size), it is already floating in a basket-like area formed by connective-tissue trabeculae and intervening lacunae filled with liquid.

7. The Appearance of the Immature Ovary of the Platypus.

In Pl. 12, fig. 2, is drawn an immature ovary measuring 3·250 × 1·0 mm. This shows remarkably well the almost amphibian character of the ovary at this stage. As was pointed out above with reference to the mature ovary, there is also to be seen in this immature specimen a cortical arrangement of oocytes; around the ovary the eggs tend to lie in a thickened area, beneath which is a space occupying the centre of the organ. This cavity is only partly filled with loose strands of connective tissue.

One is forced to look upon this peculiar structure of the immature ovary of Ornithorhynchus as a very primitive feature.

In subsequent development the cavity becomes more and more filled with connective tissue, and this, together with the growth of the cortical walls of the ovary, caused the primitive type of arrangement to be disguised and partly obliterated; but it should be pointed out that the lacunae figured on Pl. 13, fig. 4, are largely the remains of the early cavity within the gonad.

8. The Size of the Largest and Smallest Ovarian Oocytes of the Platypus.

In the adult ovary of Ornithorhynchus no oogonia are to be found; all these seem to have undergone their maturation prophases and to have become oocytes certainly long before the animal is full grown. Even in one very small immature ovary in Professor Hill's possession there were no oogonia: this ovary measured only 3 mm. in depth (see Pl. 12, fig. 2), whereas the adult ovary is at least 12 mm. in depth. Possibly during an embryonic period all the oogonial divisions, as well as the prophases of the maturation division, have taken place, so that when the animal hatches there are already formed all the oocytes which it will possess and use during its life.

This feature, with regard to the absence of true oogonia in the ovary, does not occur in forms like the frog, where numerous pockets of true oogonia exist in the ovary of the adult (vide Gatenby, **9**). Were it not for these pockets of cells which continually proliferate new oocytes, the frog would be unable to lay three to five thousand eggs for so many seasons. In the case of Ornithorhynchus and other Mammalia, the number of offspring produced is so small as not to necessitate a continuous new supply during each breeding season.

Measurements have been taken of a number of the oocytes of the smallest dimensions I could find. The smallest was 0·07 mm., the average among the smaller being 0·08 mm. In the adult ovary the smallest oocytes measured from 0·08 to 0·09 mm.

With regard to full-grown ovarian oocytes the largest I found was 4·5 mm. in diameter, not counting the theca (Pl. 12, fig. 3): 4 mm. seems an average diameter for the ovarian oocyte of

Ornithorhynchus. The one complete egg- and shell-membrane of which I examined sections was only from 4·5 to 5 mm. in diameter, though it was difficult owing to the wrinkling to make an accurate measurement (Pl. 12, fig. 1).

9. The Young Oocyte of the Platypus.

Some oocytes which had just undergone the prophases of the heterotypic division were discovered in the Flemming-fixed material; two such oocytes are drawn on Pl. 14, figs. 7 and 8. The nucleus is nearly always spherical, but occasionally irregular as shown in fig. 8; there is a well-marked nucleolus, NU in fig. 7, of the fragmented type; in some nuclei the nucleolus can be seen to be formed of two parts—a lightly-staining region, NUP in fig. 10, and a darkly-staining region, NUB. In fig. 11 the nucleolus consists of a very large darkly-staining sphere and a number of smaller pale elements; the chromatin is feebly staining and dispersed in all these nuclei.

In nearly all the young oocytes observed a centrosphere is present, CS in figs. 7 and 8; the centrosphere at this stage lies near the nucleus, often within a dent in the nuclear membrane, as in Pl. 14, fig. 8. In some cases contrioles or small granules within the centrosphere can be made out, as in fig. 8, CS. In the youngest oocytes the centrosphere may be surrounded by a cloud of granules which have been identified as mitochondria (M), fig. 7.

In older oocytes the mitochondria, as happens in all vertebrate eggs, gradually pass away from the centrosphere, and become spread out into the cytoplasm (fig. 8); they tend to collect as matted granules and filaments, particularly in the region of the periphery of the egg, and become difficult to demonstrate at and after this period.

10. On the Early Establishment of a Polarity in the Platypus Oocyte.

All the oocytes examined showed a distinct polarity, in that the nucleus had taken up a position to one side of the oocyte cytoplasm. I believe that this polarity has no relationship to the plane of the surface of the ovary, nuclei being found lying inwards, outwards, or sideways to an axis drawn directly down at right angles to the surface of the gonad.

From the material examined it is impossible to understand completely the mode of origin of the polarity in the young oocytes, but from our knowledge of many vertebrate oogonia we are aware that when in this early stage the nucleus tends to lie to one side of the cell. The polarity of the Ornithorhynchus oocyte is therefore probably established during the oogonial stage, either as the accidental result of the position of the centrosomes and centrospheres of the daughter-cells during oogonial divisions, or as a subsequent and more expressly determined movement of the oogonial nucleus within the cytoplasm, at a stage just before the inception of the prophases of the heterotypic divisions. The former is most likely.

This polarity of the oocytes persists throughout their entire growth, marking permanently the position of blastoderm and vegetative pole of the full-grown oocyte, and of the part of the egg in which the latebra will be formed.

11. Formation of Egg-membranes.

The egg-membranes on the ovarian oocyte of Ornithorhynchus are a theca (externa and interna), a follicle, and a zona pellucida.

In all the youngest oocytes that have been observed the follicle is well formed; it is shown in Pl. 14, figs. 7 and 8, FOL, and much enlarged in fig. 9. In the latter figure the follicle is seen to consist of one layer of flattened cells, overlying the substances of the oocyte (oc). In good preparations it is possible to recognize clearly a limiting or true cell-membrane around the egg-cytoplasm, OM, in Pl. 14, fig. 9. Distinct cell-walls between the individual cell elements of the follicle were generally difficult to find, but are probably always present.

In Pl. 14, fig. 12, the same region of an older oocyte is drawn. The follicle cells as such could not be identified in this preparation, but the nuclei and general cell-substance have increased greatly in size. Just at this stage a new arrangement of the individual elements of the follicle begins to take place; the nuclei dividing rapidly, soon become too large and too numerous to lie all in one row in the follicle, and gradually certain nuclei are displaced, as shown in Pl. 14. fig. 12, and ultimately a two-layered follicle results (Pl. 14, fig. 11, FOL). Two-layered the follicle remains all through its subsequent life.

Now comes one of the events most difficult to understand and interpret—namely, the formation of the zona pellucida. Possibly, however, judging from the accounts of workers who have studied other material, Ornithorhynchus presents the problem in a less difficult form, though there are some points which are still far from clear to me.

A glance at Pl. 14, fig. 9, gives one an impression of the condition of the egg-membrane (OM) at this early stage—the membrane is a true cell-wall, and nothing else at this period.

Now in Pl. 14, fig. 12, the egg is considerably older, and two new structures have appeared: one is the substance marked PZ, the other the fibrillae marked CF. The substance marked PZ is the precursor of the zona pellucida, while the fibrillae, CF, grow to form the much larger structures shown in Pl. 14, fig. 14, at CF. The fibrillae serve as connecting elements between the zona pellucida and the outer cell-membrane (OM) of the oocyte cytoplasm.

In none of the best slides I examined could I be sure that cell-walls existed at the stage drawn in Pl. 14, fig. 12, just when the pre-zona substance is becoming clearly marked. The follicle nuclei appear to lie within a syncytium, but in my mind there exists no doubt that the pre-zona material is formed in or by the follicle cells. The substance might possibly be intercellular, as described by Miss Thing, but it is certainly derived from the follicle; moreover, up to the last step in the development of the oocyte the follicle cells lie in close relationship with the zona, as in Pl. 14, fig. 13, and when the egg is extruded the naked edges of the follicle cells are left, apparently supporting the view that the zona and the follicle were previously most intimately related. This is all I can write with reference to the development of the zona.

In a well-advanced oocyte the zona and the underlying structures appear as drawn in Pl. 14, fig. 14. The zona has stained black with haematoxylin; beneath the zona is the true cell-wall of the egg (OM), which is quite thick. I call this the true cell-wall of the egg because I believe it can be traced back to the undoubted cell-wall of the earliest oocyte, marked OM, in Pl. 14, fig. 9. In Pl. 14, fig. 14, the cell-wall (OM) is connected to the zona by a large number of cortical fibrillae; these, marked CF in Pl. 14, figs. 12 and 14, probably serve the dual purpose of attaching the zona firmly to the egg, and of acting as living protoplasmic connexions between the nutrient bringing follicle and the receptive interior of the egg.

Outside the theca itself is possibly another layer of less closely packed, often obscurely defined cells, which can be recognized as a theca externa, distinguishable from the true theca, or theca interna (Pl. 14, fig. 13, TH and OSTR). The theca externa, like the true or inner theca, is formed by cells which, sympathetic to the development of oocyte, become slightly flattened and help to form a supporting and vascular capsule for the egg.

12. Yolk Formation in the Platypus.

The egg of Ornithorhynchus is macrolecithal and an extremely difficult object to section. Its yolk, like that of the frog's egg, stains densely in iron alum haematoxylin. Pl. 12, fig. 3, gives an idea of the appearance of a section stained by this method. In another paper[1] on the full-grown egg (shortly in press), in Pl. 1, fig. 1, the enormous number of yolk-granules can be noted. After fixation of the ovary in acetic acid fixatives, the formation of the yolk is seen apparently to be heralded by the appearance of a number of vacuoles beneath the periphery of the egg. These vacuoles, which are shown in Pl. 13, fig. 4, at B, are probably filled with a lipoid substance of some sort, for at this stage the egg preserved in chrome-osmium does not exhibit such vacuoles.

[1] In this paper is described the polar body formation and minute structure of the latebra of the maturing ovum.

Now at a later stage of oogenesis as seen after non-osmicated fixatives, the yolk-granules are observed to appear beneath or within the wall of vacuoles, as shown in Pl. 13, fig. 4, C. This stage is drawn at a higher magnification in Pl. 13, fig. 5; the vacuoles are at V, and lie below the non-vacuolated clear outer zone of the egg (OZ); here and there on the trabeculae between the vacuoles, but mainly beneath the vacuoles themselves, are found in all stages of development yolk-spheres, YA, YB, YC. Beneath this row of yolk-elements the egg-cytoplasm again becomes non-vacuolated, forming a distinct inner zone at this period IZ.

At a still later stage the inner zone free from yolk still persists, but smaller in extent comparatively with the rest of the egg (Pl. 13, fig. 4, D).

The individual yolk-granules may be noted to become formed within certain of the clear vacuoles. In Pl. 13, fig. 5, the vacuole at YA contained a partially formed yolk-granule, or in other words was filled *intra vitam* with yolk-substance so thin in quality as not to be firmly coagulated by the fixative, and thus gave the shrunken appearance noticed in YA and YB. The yolk-sphere at YC was older and became fixed more intensely, not undergoing shrinkage.

I feel sure that many of the yolk-granules form by additions, from the surrounding cytoplasm, to the fluid contents of the vacuoles. The latter appear first, and then their contents become richer and richer, till the yolk-granule is completely formed. From the material available I was unable to say whether the mitochondria take any part in yolk formation.

13. Formation of Latebra.

In Pl. 13, fig. 4, are several stages in the formation of the latebra; in fig. 4, B, the oocyte cytoplasm presents a ring of vacuoles which divide the egg into two parts, an outer (B) and an inner (BL); the latter forms the main part of the latebra. The latebra is that part of the inner region of the egg where no coarse yolk-granules are ever formed.

In Pl. 13, fig. 4, C, a later stage is shown; the yolk-granules have begun to form beneath the layer of vacuoles, and the clear space inside will form the body of the latebra. Reference to Pl. 13, fig. 5, will show that not all the inner non-vacuolated area (IZ) forms latebra, for at YA to YC is an area in which yolk-granules appear in this region.

In Pl. 14, fig. 13, a still later stage is drawn; this oocyte is interesting because it shows how the formation of coarse yolk-granules (OZ) never takes place in the region beneath the nucleus (NU). It is just in this region beneath the nucleus that the cone of protoplasm (CP in Text-fig. 1) and the upper part of the latebra meet to form the so-called Nucleus of Pander.

The latebra, at a later stage, is shown in Pl. 13, fig. 4, D. At UAL the neck of the latebra is distinguishable and the substance of the latebra itself (BL) has become very vacuolated, as indeed has the whole egg, especially after preparation in acetic acid corrosive fixation.

In another forthcoming paper the appearance of the latebra in the fully-formed egg has been described. It should be noted that the latebra is not formed by the path left by the movement of the nucleus, as is thought by some to be the method of origin of this structure.

14. A Fully-formed Egg (diameter 4 mm.).

In Pl. 12, fig. 1, is a figure, only slightly diagrammatic, of a fully-formed egg of the duck-billed Platypus. On the outside is the thin shell-membrane (SM), which owing to the contact of the fixatives had become somewhat bent and irregular. Beneath the membrane is a layer of albumen or white (W), which is seen in finished sections as a flocculet lightly-staining substance. The egg-white has been pushed out of place on one side by the bending of the shell-membrane.

The rest of the egg is formed of the ovum (oocyte) proper. It is bounded on the outside by a very thin membrane (Z) called by Caldwell the vitelline membrane, and which I believe to be the zona. In the egg drawn are two distinct areas, an outer (OZY) and an inner (IRY) yolk-zone.

The latebra passes up from the centre of the egg (LZL) to the region generally called the Nucleus of Pander (UAL) beneath the blastodisk (BD), in which the nucleus (NU) is situated.

For further details of the egg proper the other paper should be consulted. The neck of the latebra and the region of the Nucleus of Pander has been described therein more fully.

Faithful drawings of the shell-membrane and its underlying areas have been made by Caldwell, and will be found in his paper.

The average size of the fully-formed egg of the platypus is about 4 mm.; but, as Wilson and Hill have pointed out, it soon absorbs liquid from the uterine walls, and grows to 12 or 14 mm. at the time of laying.

15. Note on Spermatogenesis.

Among Professor J. P. Hill's material were some sections of Ornithorhynchus testis, and in Pl. 13, fig. 6, I have given a drawing of a part of one semeniferous tubule and some interstitial tissue. Very good figures of the spermatozoa have previously been made by Retzius and Benda.

Two of the most striking facts about the histology of the platypus testis are the large size of the cells of the interstitial tissue (INT) and the remarkable development of the Sertoli cells (SC). The latter seem to be derived directly from the basement cells or primitive spermatogonia, and I could find nothing suggestive of the presence of any kind of Sertoli cell determinant as described by Montgomery for man. In the platypus the primitive spermatogonium probably becomes a Sertoli cell merely in sympathy with the development of a group of spermatocytes above it. At SPT (lower) are a group of spermatocytes nearly full grown, and beneath them, at YSC, is a cell which is in the same series as the primitive spermatogonia above and below (SPG), but which is hypertrophying step by step with the group of spermatocytes near by. At SC is a Sertoli cell ready for the fixation of the spermatids (SPD^1) which is just beginning, and at SPD^2 is a Sertoli cell with the later spermatids all attached. At SPZ is a group of ripe sperms attached to a fully-formed Sertoli cell. The sperms are not spatulate, but resemble those of reptiles and birds, except that the cytoplasmic part is relatively shorter.

16. Discussion.

Probably the most interesting fact ascertained by an examination of this material of the Platypus is the presence of a large hollow cavity in the young ovary. This is undoubtedly a primitive character, which is noticeable even in the adult ovary, in the form of numerous lacunae throughout the stroma of the ovary.

The stroma of the ovary of the Platypus evidently appears early as a number of separate chords of cells which probably grew into the hollow sac-like ovary at a late stage of embryonic history. The ovary of the original vertebrate seems to have been a sac-like structure, the stroma being a new formation; the cells which in Ornithorhynchus constitute this loose stroma seem to have been formed by a retro-peritoneal invasion, but as has been pointed out above, they never quite fill the cavity even of the adult ovary.

The egg of Ornithorhynchus is discharged from the ovary in quite a different way from that of the placental mammal. In the latter the oocyte, with a corona of follicle cells, breaks loose from the glomus proligerus and the release of the egg from its cellular bed involves only part of the follicular elements. In the case of the egg of the Platypus breakage involves the entire follicular layers, as in the case of the frog's ovary, and no liquor folliculi is present or takes part in the expulsion of the egg.

The formation of the yolk resembles that of the bird described by Van Durme, and the latebra forms in the same manner. Some, at least, of the yolk-spheres are formed as in birds, i. e. by the appearance of watery vacuoles in the ground cytoplasm, and the subsequent loading up of the contents of these vacuoles with fatty and proteid substances, thus constituting coagulable and ' solid ' yolk-spheres.

The zona appears to be formed from a substance which is intracellular at first; but it must be admitted that the matter was difficult to decide. In none of the preparations could distinct cell-walls be found in the follicle at the period when the zona substance was beginning to appear. There is no doubt in my mind that the substance of the zona is formed in direct relationship to the cells of the follicle, and the cytoplasm of the egg probably takes merely a secondary or stimulatory part in the production of this important membrane.

The mitochondria, so far as they could be followed out, act in the same manner as in both the fowl and the frog, and the young oocyte contains the same formed elements as that of the fowl, i. e. sphere, centrioles, and cloud of mitochondria.

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Explanation of Lettering.

A, youngest oocyte found. AT, area of attachment of ovary to body-wall. B, oocyte at beginning of formation of latebra. BD, blastoderm. BL, inner region of egg, which will form part of latebra. BV, blood-vessel. C, egg at stage of beginning of yolk formation. CA, cavities in ovary. CF, cortical fibrillae, beneath zona. CH, chromatin. CS, centrosphere. DY, dark-staining yolk. FOL, follicle of egg. GE, germinal epithelium. INT, interstitial tissue of testis. IRY, inner region of yolk. IVZ, inner vacuolated zone of egg. IZ, innermost zone of oocyte. LZL, lower zone of latebra. M, mitochondria. NU, nucleus. NUB, darkly-staining nucleolus. NUP, faintly-staining nucleolus. OC, cytoplasm of oocyte. OFOL, outer limiting membrane of follicle. OM, cell-wall of oocyte. OO, oocyte. OOX, small oocyte compressed by growth of a larger one. OSTR, outer region of theca (theca externa). OZ, outer or peripheral zone of oocyte. OZY, outer zone of yolk. PY, pale yolk. PZ, pre-zona, or substance which forms zona pellucida. SC, Sertoli cell of testis. SM, shell-membrane. SPD, 1 and 2, two stages of spermatids. SPG, spermatogonium. SPT, spermatocyte. SPZ, spermatozoon. TH, theca (interna). TR, ovarian trabeculae. UAL, upper area of latebra. V, vacuole. W, egg white. X, material formed probably by degeneration of oocytes. XY, enigmatic plasmatic body in young oocytes. YA, YB, YC, stages in formation of yolk-spheres. Z, zona.

DESCRIPTION OF PLATES.

Plate 12.

Fig. 1.—Fully-formed egg of Ornithorhynchus paradoxus, in vertical section. Shows latebra, yolk, albumen, and shell-membrane.

Fig. 2.—Transverse section of a young ovary showing cavity (CA) and loose trabeculae (TR), and cortically arranged oocytes (OO).

Fig. 3.—Fully-developed ovary of Ornithorhynchus, oocytes blacked in. Cavities in stroma (CA) cross-hatched.

Plate 13.

Fig. 4.—Part of adult ovary more highly magnified showing oocytes in different stages. The numerous cavities in the stroma are evident, and several stages in the formation of the latebra are given (B, C, D). In the egg D, the follicle is only put in below.

Fig. 5.—Part of the egg at an early stage of yolk formation, as in fig. 4, C. Cortical vacuoles and deeper yolk formations are shown.

Fig. 6.—Part of the testis of Ornithorhynchus. For description see p. 492 of text.

Plate 14.

Figs. 7 and 8.—Two of the youngest ooeytes or Ornithorhynchus, showing sphere and mitochondria.

Fig. 9.—Edge of young egg showing relationship of follicle to cell (oocyte) membrane.

Fig. 10.—Egg at time of formation of pre-zona (PZ) follicle, one-layered.

Fig. 11.—Later stage, zona formed, follicle two-layered.

Fig. 12.—Follicle and part of egg at stage little later than in fig. 10, showing pre-zona substance apparently within follicle wall. Two layers of nuclei just forming in follicle.

Fig. 13.—Detail of later egg, showing membranes. Mitochondria at M.

Fig. 14.—Cortex of later egg showing arrangement of layer beneath the zona.